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CERTIFICATE OF INTERNSHIP COMPLETION

This is to certify that **Ms. Sonia Saha**, a student of the **Department of Biotechnology, Sister Nivedita University, Kolkata**, has successfully completed her internship project entitled- **"Studies on Deconstruction of Lignocellulosic Biomass by Pigmented Indigenous Bacterial Monoculture – Structural and Physicochemical Analysis"**, under the supervision of **Dr. Indrani Paul**, Assistant Professor, Department of Biotechnology, School of Biotechnology and Biosciences, Brainware University, Kolkata.

The internship was conducted at the **Department of Biotechnology, Brainware University, Kolkata,** from 1st March to 31st May, during which Ms. Saha demonstrated sincerity, dedication, and a keen interest in research activities.

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DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "Studies on Deconstruction of Lignocellulosic Biomass by Pigmented Indigenous Bacterial Monoculture - Structural and Physicochemical Analysis" in partial fulfilment of the requirements for the award of the degree of Masters of Biotechnology in Sister Nivedita University is an authentic record of my own work carried out during the period from 10.02.2025 to 31.05.2025 under the supervision of Dr. Indrani Paul Department of Biotechnology, Brainware University, Kolkata.

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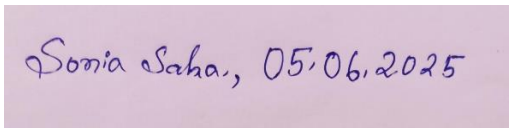

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ABBREVIATIONS

Serial no	Abbreviation	Full form
1.	AIL	Acid insoluble lignin
2.	ASL	Acid Soluble Lignin
3.	CPC	Chemically Purified Cellulose
4.	DNS	Dinitrosalicylic Acid
5.	FTIR	Fourier Transform Infrared Spectroscopy
6.	LCB	Lignocellulosic Biomass
7.	SCB	Sugarcane Bagasse
8.	SmF	Submerged Fermentation
9.	UV-Vis	Ultraviolet-Visible Spectroscopy
10.	YPMSM	Yeast Peptone Mineral Salt Medium

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Studies on Deconstruction of Lignocellulosic Biomass by Pigmented Indigenous Bacterial Monoculture- Structural and Physicochemical Analysis

1. Abstract-

Sugarcane bagasse is an abundant agro-industrial lignocellulosic biomass with great potential as a renewable raw material for sustainable bioconversion. In this study, we investigate the microbial degradation of sugarcane bagasse by a naturally occurring, pigmented bacterial strain (ROC1) isolated from soil. Bagasse is rich in cellulose, hemicellulose and lignin and is a good candidate for microbial treatment to produce value added products. The bagasse was pretreated with sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4) to pretreat the bagasse to enhance the bioavailability of its fibrous components by breaking down the rigid structure. Subsequently, the treated biomass was used as the sole carbon source in a submerged fermentation setup with yeast-peptone mineral salt medium. Colonization and rapid proliferation of the bacteria was confirmed by monitoring bacterial growth through colony forming unit (CFU) counts and optical density. The degradation efficiency was assessed by DNS assays and UV-Vis spectrophotometry which confirmed significant breakdown of lignin, cellulose and hemicellulose. Interestingly, the bacterial strain also produced a yellow orange pigment which was identified as riboflavin (vitamin B2) by FTIR and UV-Vis spectroscopy. FTIR further structural analysis showed significant chemical changes in the sugarcane bagasse matrix after bacterial treatment. These results indicate that native soil bacteria can degrade lignocellulosic waste and produce valuable bioproducts simultaneously. Sugarcane bagasse is positioned in the study as a waste byproduct, but also as a promising substrate for microbial fermentation and green bioprocessing applications.

Keywords:-[sugarcane bagasse, lignocellulosic biomass, bacterial monoculture, riboflavin, biopigment, submerged fermentation, biodegradation, waste valorization]

2. INTRODUCTION -

Lignocellulosic biomass (LCB) comes from plant material, is plentiful and renewable and contains three primary polymers: cellulose, hemicellulose and lignin (Zoghlami 2019). The structure of LCB suggests it will be a good and economical source of fermentable sugars and other useful biochemicals. Even so, biodegrading LCB is tricky because it involves many different types of enzymes (Tolalpa, 2017). Out of all lignocellulosic residues, sugarcane bagasse (SCB) has stood out as a favorable option for bioconversion techniques. SCB is a waste material that forms when sugarcane juice is pressed and there is a lot of it in places with tropical and subtropical climates. For this study, the SCB came from a local juice vendor in Barasat and was used because it was cost-friendly and easily available. The major building blocks of SCB are cellulose, hemicellulose and lignin, of which Xylan accounts for most of the hemi cellulosic part, while glucan includes both cellulose and hemicellulose (Vieira., 2019). Researchers isolated a gram-positive, pigmented bacterium from a soil sample in Kolkata, India and used it to help microbes degrade SCB. According to Nunes (2019), the decomposition of cellulose, hemicellulose and lignin in LCBs is not the same for every microorganism due to their different enzymatic and biochemical skills. A number of bacterial strains are recognized for breaking down lignocellulose and producing riboflavin (vitamin B2) which is a commercial product (Dzanaeva., 2024). Among its many jobs, riboflavin helps by making FMN and FAD which are important parts of cellular metabolism. They are necessary for important chemical changes during the metabolism of carbohydrates, lipids and proteins. While riboflavin was synthesized by chemical means previously, it is now made more often by fermentation since it is cost-friendly and environmentally sustainable. Significantly, Bacillus subtilis is used in industrial riboflavin manufacturing, but the strong cellulolytic abilities *of B. coagulans, B. licheniformis and B. cereus* enable these strains to degrade cellulose and hemicellulose (Kumar, 2024*). Thermomonospora fusca* and *Cellulomonas fimi* together play important roles in breaking down lignocellulose. The composition of sugarcane bagasse is 45-50% cellulose, 25-30% of hemicellulose, and 25% lignin (S. Niju 2019)

Because SCB is a waste product from farming, it can be used in various industries thanks to its high amount of cellulose. The objective of this investigation was to separate out cellulose nanocrystals (CNCs) and chemically purified cellulose (CPC) from SCB to show its potential use in biomedical applications (Evans, 2019). An initial microbial pre-treatment step in YPMSM was applied to SCB,

as it contains important phosphates, nitrates, sulphates and chlorides that microbes need without added carbon (Park, 2020). Many biodegradation experiments use YPMSM because it promotes microbial growth and obliges them to feed on lignocellulosic materials only (Kaur, 2021). 12–16 hours before starting, a nutrient broth culture of each bacteria was incubated at 36°C and shaken constantly at 120 rpm. The bacteria were added at a 5% concentration to the YPMSM with SCB and incubated at 30°C for 120 hours, with mixing done every 12 hours. R. Khaneja (2009) also considered the effect of waste biomass on pigment production. Submerged fermentation was selected over solid-state fermentation because controlling temperature, pH and oxygen is easier with submerged fermentation (Pyter, 2022). Solid-state microbial fermentation ensures equal bacterial growth, increases the amount of produced products and provides an easy scaled process for industry purposes. What's more, SmF speeds up extracting useful items since products can be extracted from the liquid phase without complications. To evaluate the results of microbial treatment on SCB, analytical and biochemical methods were used. To improve the breakdown of biomass, solutions of sodium hydroxide (NaOH) and sulfuric acid (H2SO4) were used according to methods described in previous investigations (Anwar, 2014). Biomass that had been treated was studied using FTIR spectroscopy and UV-Visible spectrophotometry to understand both its structure and its a chemical changes (Majumdar, 2020).

3. Objective

- To perform submerged batch fermentation of bacterial strain in SCB containing Yeast-peptone mineral salt media
- To assess the LCB deconstruction potential of a given bacterial monoculture – strain ROC1
- To extract and analyse the coloured bio pigment produced by the strain.

4. Review of literature:

According to Aruldass *et.al.*(2016) Bacterial pigments showed potential in various applications but the pigment production is limited due to high cost. This study reports on the optimization of yellowish-orange pigment production by *Chryseobacterium artocarpi C*ECT 8497 in liquid pineapple waste medium using response surface methodology (RSM). Culture conditions affecting pigment production were optimized statistically in shake flask experiments. Liquid pineapple waste, l-tryptophan and potassium phosphate (KH_2PO_4) were the most significant variables affecting pigment production. Central composite design (CCD) and RSM analysis were adopted to investigate the interactions between variables and determine the optimal values for maximum pigment production.

According Majumdar *et,al.* (2020) This study explored Paper mill sludge (PMS) and sugarcane bagasse (SCB) as inexpensive substrate for *Planococcus* sp. TRC1 mediated valuable β-carotene production and residual treated biomass as value added crystalline cellulose source simultaneously. Both biomasses supported significant bacterial growth reaching highest yield 38.54 ± 1.4 mg/g on PMS (36 h) and 47.13 ± 1.9 mg/g (48 h) on SCB in solid state fermentation. Luedeking-Piret model revealed growth associated production with α and much lower β values of 5.18 and 0.24 for PMS and 4.5 and 0.165 for SCB.

According to Nunes *et, al .(*2020) In this work, sugarcane samples were acquired, and the process of obtaining bagasse was simulated. Subsequently, the bagasse was dried and heat-treated at 200 and 300 °C to simulate the over-drying and torrefaction process. Afterward, product characterization was performed, including thermogravimetric analysis, elemental analysis, calorimetry, and energy densification. The results showed significant improvements in the energy content, from 18.17 to 33.36 $MJ \cdot kg^{-1}$ from dried bagasse to torrefied bagasse at 300 °C, showing that despite high mass loss, there is potential for a future value-added chain for this waste form, since the increment in energy density could enhance its transportation and use in locations far off the production site.

According to Petridis *et, al*. (2018) The plant cell wall biopolymers lignin, cellulose and hemicellulose are potential renewable sources of clean biofuels and high-value chemicals. However, the complex 3D structure of lignocellulosic biomass is recalcitrant to deconstruction. Major efforts to overcome this recalcitrance have involved pretreating biomass before catalytic

processing. This Perspective describes recent work aimed at elucidating the molecular-level physical phenomena that drive biomass assembly. These are at play in commonly employed aqueous-based and thermochemical pretreatments. Several key processes have been found to be driven by biomass solvation thermodynamics, an understanding of which therefore facilitates the rational improvement of methods aimed at the complete solubilization and fractionation of the major biomass components.

According to Dar *et.al.* (2018) The present study demonstrates the enrichment, isolation and identification of cellulose degrading bacteria from the gut of cotton bollworm, Helicoverpa armigera. The isolate MD21 was identified as Klebsiella sp. MD21 (MG367463) based on 16S rRNA gene sequencing. The isolated bacterium, Klebsiella sp. MD21 secreted many hydrolytic enzymes particularly xylanase and β-glucosidase with highest activities of 276.71 ± 0.98 and 78.45 ± 0.10 IU/ml extract respectively. Scanning electron microscopy of treated filter paper and saw dust revealed structural alterations caused by *Klebsiella* sp. MD21 leading to hydrolysis of cellulosic waste into reducing sugars (2256 $\mu mol\ ml^{-1}\ min^{-1}$) which could be used for the growth of bacteria. X-ray diffraction (XRD) pattern showed an increase in crystallinity index from 58.78% in native biomass to 60.10% of the treated biomass due to hydrolysis of amorphous regions present in the saw dust.

According to Liang *et.al* (2018) Recalcitrance limits biomass application in biorefinery. It is even more so when toxic chlorophenols are present. In this study, we screened a microbial consortium, OEM2, for lignocellulose deconstruction and chlorophenols detoxification through a short-term and efficient screening process. Microbial consortium OEM2 had a good buffer capability in the cultivation process and exhibited a high xylanase activity, with over 85% hemicellulose degradation within 12 days. Throughout the treatment process, 41.5% rice straw decomposition on day 12 and around 75% chlorophenols (MCP, 2,4-DCP, 2,4,6-TCP) removal on day 9, were recorded. Moreover, Fourier translation infrared spectroscopy (FTIR) analysis indicated that chemical bonds and groups (eg. hydrogen-bond, β-1,4 glycosidic bond, lignin-carbohydrate cross-linking) in the rice straw were broken. Cuticle and silica layer destruction and subsequent exposed cellulose fibers were observed by scanning electron microscopy (SEM). Microbial consortium OEM2 diversity analysis by 16S rRNA gene sequencing indicated that *Proteobacteria* (41.3%) was the most abundant phylum and the

genera *Paenibacillus* and *Pseudomonas* played an important role in the lignocellulose decomposition and chlorophenols detoxification.

According to Jesus *et.al* (2019) The conversion of biomass to biofuels presents a solution to one of the largest global challenges of our era, climate change. A critical part of this pipeline is the process of breaking down cellulosic sugars from plant matter to be used by microbes containing biosynthetic pathways that produce biofuels or bioproducts. In this inquiry-based course, students complete a research project that isolates cellulase-producing bacteria from samples collected from the environment. After obtaining isolates, the students characterize the production of cellulases.

According to Ahmed *et.al* (*2018*) Lignocellulosic biomass is the most abundant biomaterial on Earth and holds enormous potential as a source of fermentable sugars for cost-effective biofuel production. The main obstacle is the intractable and convoluted structure and composition of lignocellulosics which are mainly made of lignin, cellulose and hemicellulose. However, nature has equipped microbes with enzymes that can deconstruct lignocellulose and release fermentable sugars. In this study, we selected bacteria (belonging to 6 different genera) from nature that had been grown in minimal culture media with cellulose or lignin as the sole carbon source, and assessed their lignocellulolytic activities in qualitative and quantitative assays. Through sequence analysis of the 16S rRNA gene, we discovered a novel lignocellulolytic bacterial species, *Chryseobacterium gleum*, capable of degrading both cellulose and lignin. In addition, a few other bacterial species previously known to degrade either cellulose or lignin were found to have lignocellulolytic activities. The majority of these bacterial species were found to produce extracellular lignocellulolytic enzymes. Lignocellulolytic bacteria identified in this study may serve as sources of genes/enzymes which might find applications in biofuel production and other industrial applications.

5. Material and Methods

5.1 Materials-

All material used in the study were laboratory grade. Media components were purchased from Himedia , SRL pvt.ltd , Merck (Germany). Glass ware used from Borosil, plastic (Tarson), SCB was purchased from local market .

5.2 Methods

5.2.1 Pretreatment of Sugarcane Bagasse: -

The sugars of the sugarcane bagasse used in this study were supplied by several local juice vendors. Suitable SCB For the study needed to be white to light yellow in color and free from fungal contamination, all of which were accounted for. I cleaned the collected SCB with distilled water three times to take out any dust or dirt. Small pieces of SCB were made in order to make drying easier, Then the washed SCB was baked in an oven at 70 ℃ for 24 hrs before it was ground in a mixer grinder. The SCB granules were all the same size after being passed through a 0.5 mm sieve. Keep this primary material for further reviews. Novel uses for chitin include its role as a source of cellulose which excites many for its benefits in the biomedical sector. I separated cellulose nanocrystals (CNCs) and chemically purified cellulose (CPC) from sugarcane bagasse (SCB) during my present study. (Evans, 2019)

5.2.2. Submerged Fermentation for bacterial Growth on SCB

Most of the time, SmF is preferred for using SCB, as the SCB is soaked in a liquid medium which allows for simpler control over temperature, pH and oxygen. Tests were run in batches to check if the bacteria could use SCB as both a substrate and a source of carbon. Afterwards, prepare YPMSM (which include 0.5 % yeast extract, 0.5% peptone, KH_2PO_4 a 1.2 g/l, K_2HPO_4 4.8g/l, $MgSO_4$ 0.25 g/l, $FeSO_4.7H_2O$ 0.0025 g/l, $Cacl_2,2H_2O$ 0.026 g/l and NaCl 5 g/l) and add 500 µl of micro solution to the prepare The selection of 20% SCB in the media came from many research studies showing that was the balance required to enable the bacteria to grow in YPMSM. A solid culture of bacteria was used to inoculate the samples prepared in SCB-YPMSM. Routine withdrawals of culture samples were done all the way up to when the stationary phase started. All sample removals were passed through Whatman filter paper to remove any sugarcane bagasse fragments, so they would

not affect the setting of biomass. To free any bacteria connected to the SCB substrate, the samples were washed twice with equal amounts of sterile distilled water. The filtrate was then centrifuged at 8000 rpm for 15 minutes to pack down the bacterial material. Phosphate-buffered saline (PBS) was used to resuspend the pellets and their optical density (OD) was determined at 600 nm on a UV-Vis spectrophotometer.

5.2.3. Growth Kinetics: -

Yeast peptone Mineral salt Media (YPMSM) containing SCB 20% was prepared in 2 litre conical flasks containing 500ml media and sterilized by autoclaving. overnight activated 5% bacterial culture was inoculated and fermentation was carried out in submerged state at 30ºc with an agitation speed of 120 rpm. Cell free supernatant was prepared by withdrawal of culture, followed by separation of supernatant by centrifugation at 8000rpm for 15mins. The culture supernatant was tested for emulsification activity at frequent intervals and surface tension of the supernatant was measure. YPMSM containing SCB is incubated for 7 days and in these time period CFU counting was done using the SCB sample.

Isolation and Cultivation of Bacterial Strain: -

Day 1-An aliquot containing 500 µL of environmental sample was added to a nutrient agar plate following the spread plate method. The plate was next put in the incubator at 36°C for 24 hours so bacterial colonies could first grow.

Following incubation on day 2, the cay sample was diluted in series to allow counting of the colonies that form from each dilution. A dilution of 10^{-6} was prepared, plated and incubated at 36°C for a period of 24 hours.

Day 3-Fresh material was made and put directly onto two sets of agar plates. The first plate got the sample with no dilution and the second got the 10 to the minus second dilution. Both plates were held at 36°C for 24 hours so the bacteria could grow.

During day 4, the sample was diluted by a factor of 1 followed by its plating onto a nutrient plate. The plate remained at 36°C for an added 24 hours.

For Day 5, another dilution using a 10^{-4} factor was carried out on the plate which was left at 36°C for monitoring of bacterial colonies.

5.2.4. Assessment of LCB Deconstruction: -.

To determine if the selected bacterium could use sugarcane bagasse lignin, the amount of lignin in the material needed to be measured before and after bacterial treatment. SCB was placed in Yeast peptone mineral salt medium (YPMSM) and inoculated using activated bacterial cultures. The setup was cultured underwater at 30 °C, with 120 rpm shaking for 96 hours. Both adenylate acid-treated and untreated SCB samples were washed with distilled water several times to remove debris and any remaining bacterial cells. Both sets of biomass were air-dried after cleaning and their lignin content was determined. The analysis separated all forms of lignin, including alkali lignin, acid-insoluble lignin and acid-soluble lignin, to determine the amount of lignin break down by the bacteria.

To determine alkali lignin content, SCB was boiled in boiling water for four hours to remove the soluble impurities and the insoluble residue was then filtered. After that, I added 15% sodium hydroxide to the residue at a ratio of 1:15 or 1:18, allowed the mixture to stand for four hours to loosen lignin, then filtered it. Because the liquid had a brown color, we knew alkali lignin was dissolved. OD readings were taken for this lignin solution at 465 nm and these were used to calculate the percentage of alkali lignin degradation using a known equation.

$$\frac{OD\ of\ untreated\ SCB - OD\ of\ treated\ SCB}{OD\ of\ untreated\ SCB}\ X\ 100$$

To determine the acid-soluble and acid-insoluble lignin content, To find the lignin content, we employed acid hydrolysis on one gram of sugarcane bagasse (SCB). Eighteen grams of ammonium nitrate in 10 mL of 72% sulfuric acid were left at room temperature for six hours. Every half an hour, the mixture was gently shaken to ensure both the acid and the solution mixed properly. After the first application, 84 mL of distilled water were mixed in with the acid to dilute it and the solution was autoclaved at 121 °C for proper hydrolysis. Autoclaving finished, the hydrolyzed mixture was left to cool at room temperature before passing through a filter to divide the substance into liquid and solid. The extracted filtrate was measured with a spectrophotometer at 320 nm to determine the acid-soluble lignin (ASL) it contained. What was left was collected, dried and measured to discover the acid-insoluble lignin (AIL) fraction. Both untreated and bacterial samples of SCB were tested using this procedure. To measure lignin degradation, the lignin level in the treated samples was examined alongside the level seen in the untreated controls. Lignin degradation percentages were

worked out using formulas suitable for both ASL and AIL measures to understand how biological treatment worked.

$$\%\ of\ acid\ sol\ lignin\ degradation = \frac{OD\ of\ untreated\ filtrate - OD\ of\ treated\ filtrate}{OD\ of\ untreated\ filtrate}\ X\ 100$$

$$\%\ of\ acid\ insol.\ lignin\ degradation = \frac{Dry\ wt.\ of\ untreated\ residue - Dry\ wt.\ of\ treated\ residue}{Dry\ wt.\ of\ untreated\ residue} X\ 100$$

To determine Hemicellulose and cellulose content- The breakdown of cellulose and hemicellulose from sugarcane bagasse (SCB) was also evaluated in addition to lignin. For estimating hemicellulose, 1 gram of SCB was reacted with 150 ml of 500 mmol/L NaOH and left to boil for three hours. After it had boiled, the mix was set aside to cool at room temperature before being filtered. The residue was washed many times with distilled water until the water became neutral. Once the biomass was washed, it was put into a hot air oven set at 105 °C until its weight no longer fluctuated. The weight difference between what was left after treatment and what was there originally was related to the amount of hemicellulose removed. A comparison of the hemicellulose content in treated SCB with that in untreated SCB was made to assess hemicellulose degradation. After the calculation, we could evaluate how much of the hemicellulose was broken down by both biological and chemical means.

To determine sugar content within SCB-

Besides other parts, the sugarcane bagasse (SCB) consists of cellulose and hemicellulose polymers, neither of which naturally shows reducing properties. But the products gained from hydrolysis—monosaccharides such as glucose made from cellulose and xylose, arabinose, mannose and others from hemicellulose—are all forms of reducing sugars. Because of this, tracking shifts in the sugar content of biomass provides an alternative way to understand when cellulose and/or hemicellulose are being broken down by microbes. Less reducing sugar present following bacterial treatment suggests that the microorganism used cellulose and/or hemicellulose as its energy source. We measured the total sugar content in both treated and untreated SCB, comparing them to evaluate this. Comparing the bacteria helps us understand how much of the polysaccharide components in

lignocellulosic biomass can be used by the bacteria. The DNS Assay for Reducing Sugar Estimation was carried out. The DNS assay is a common way to measure glucose and similar reducing sugars that are produced by breaking down sugarcane bagasse (SCB). The method uses 3,5-dinitrosalicylic acid (DNS) which changes color after reacting with reducing sugars and can be measured at 540 nm. For the DNS reagent, mix together 1 g of 3,5-dinitrosalicylic acid, 30 g of sodium potassium tartrate, 0.5 g of sodium sulphate, 2 g of NaOH and 100 mL of distilled water. Extra material consisting of a glucose stock solution (1 mg/mL) prepared in distilled water is required. A series of glucose test tubes are prepared, each with a different amount of glucose—0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL—and each mixed with 1 mL of DNS reagent. Just like before, a test tube gets 1 mL of the hydrolyzed SCB sample plus 1 mL of DNS reagent added. Heat all the tubes in a water bath when it is boiling for 5–10 minutes, till they develop a reddish-brown color, then leave them at room temperature. After the tubes cool, they are each allowed to be mixed up with 10 mL of distilled water and read by a spectrophotometer at the absorption wavelength at 540 nm. To find the percentage of reducing sugars in the SCB, you make a standard curve with glucose standards.

5.2.5. Physiochemical Characterization of Deconstructed SCB:

- **Ultra violet – visible spectroscopy (UV-vis studies)**

Characterizing colloidal suspensions of nanomaterials often relies on using UV-Visible spectroscopy. This method is respected because it is simple, fast, sensitive and always reliable. In this technique, wavelengths between 200 and 400 nm in the ultraviolet and 400 to 800 nm in the visible part of the spectrum cause electrons in molecules to move from one state to another. During these transitions, the excitation of non-bonding (n) or pi-bonding (π) electrons takes place when moving these electrons from their present, low-energy state to a necessarily higher level. Measurements in UV-Visible spectroscopy are based on Beer–Lambert's law which connects absorbance (A) to concentration (C), the length of the cuvette (L) and molar absorptivity (ε), with the equation $A = \log(I_0/I) = \varepsilon CL$. The Shimadzu UV-1800 spectrophotometer was used for measurements in this study and delivers dependable results between 190 and 1100 nm. Yet, ultraviolet radiation with wavelengths less than 200 nm is commonly excluded because of effects from oxygen. Using this method, it becomes possible to assess the formation, stability and optical aspects of nanoparticles from their absorbance spectrum.

- **Fourier Transform Infrared Spectroscopy (FTIR Studies)**

FTIR analysis offers a clear picture of any structural changes that occurs in lignocellulosic biomass after treatment by showing the sorts of functional groups that are present. If the primary peaks alter in a visible way or new peaks develop, it could mean that new groups are being made from existing groups in the biomass. FTIR research showed that bacterial treatment generated structural alterations in both the SCB and its separated lignin and hemicellulose fractions. We created fine powder out of dried SCB and combined it with KBr in a 10:90 ratio. Then we used FTIR spectroscopy in the Therm Nicolet iS10 instrument to study it.

5.2.6. Biopigment extraction:

To make and harvest microbial vitamins, a fermentation system was developed using YPMSM medium with 20% (w/v) of sugarcane bagasse added as the carbon source. Erlenmeyer flasks of capacity 1 liter were used and each one held 500 mL of liquid medium. All the medium was then sterilized by means of autoclaving. Five percent of the overnight-grown bacteria was aseptically put into the culture after sterilization. The fermentation was done while the sample was submerged, at 30 °C and continuously agitated at 120 rpm for 7 days. After the set period of incubation, all the culture was filtered to eliminate solids and several minutes later, a bit of the culture broth was centrifuged at 8000 rpm for 15 minutes to divide bacterial cells from the upper part. The mass of biomaterial was then processed to obtain the vitamins. At first, the culture was spun in the centrifuge at 8000 rpm for 10 minutes at 4°C to condense the cells. The extracted pellet was cleaned and transferred to methanol which is a suitable solvent for water- and fat-soluble vitamins. Once the mixture had been mildly stirred, it was spun in the centrifuge to clear away residual things found around the cells.The sonication method after methanol treatment broke apart the bacteria and permitted extraction and release of the vitamins. The treatment made the bacterial membranes weaker which boosted our ability to collect components. Lysate that had gone through sonication was once more centrifuged at 8000 rpm for 10 minutes after which the supernatant containing the vitamins was taken out and stored at a low temperature for measuring using UV-Vis spectrophotometry or HPLC. It points out that using SCB supports biofilm formation and encourages microbes to generate many valuable chemicals, for example vitamins.

5.2.7. Characterization of the Biopigment:

The yellow-orange pigmented microorganisms in this study made a bio-pigment called Riboflavin, which is also known as vitamin B2. Vitamins are important organic micronutrients that living things need in small amounts to work properly. Riboflavin is a vitamin that dissolves in water and is necessary for cellular metabolism. All green plants and most microorganisms make it on their own. It is very important for the growth, reproduction, and general health of people and animals. Riboflavin is a biological precursor of two important coenzymes: flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These coenzymes take part in a number of oxidation-reduction (redox) reactions that are essential for breaking down carbs, lipids, proteins, and ketone bodies, which makes a big difference in how much energy is made. Riboflavin also helps turn tryptophan into niacin and turns on other B-complex vitamins, such as B6 and B9. It also makes it easier for iron to be absorbed and moved around. There are two ways to make riboflavin on a large scale: chemical synthesis and microbial fermentation. The second method is better for the environment, more sustainable, and more cost-effective.The bacterial synthesis of riboflavin makes the production process easier, making it useful for a wide range of business uses. The main thing that this study used to measure riboflavin was how well it dissolved in water. There are a lot of studies about riboflavin's solubility in water, but not many about how to use this property in analytical methods. We came up with and tested a UV-Visible spectrophotometric method that uses water-based solvent systems to correctly measure riboflavin levels. Fourier-transform infrared (FTIR) spectroscopy was also used to confirm the pigment's chemical identity. FTIR analysis found common functional groups including O–H, C=O, and C–N, which proved that riboflavin was in the bacterial sample.

6. RESULT AND DISCUSSION:

6.1 Pretreatment of SCB:

Sugarcane bagasse (SCB), which is a waste product from farming and industry, was employed as a raw material in my current research to manufacture cellulose products that are worth more. We only picked SCB that was white to light yellow and didn't have any fungus on it so that everything would be the same and clean. The SCB was significantly more stable and had a greater surface area after being properly preconditioned. For the best extraction outcomes, both of these things are quite necessary. The SCB was ground up into a fine powder and then put through a screen to make particles that were less than 0.5 mm. This rendered the surface smooth and able to react with chemicals. It was easier to get at the lignocellulosic matrix at this stage of refining, which meant that more cellulose components were needed. We checked the material with our eyes and hands to be sure that all the dirt and other contaminants had been entirely eliminated. It was now ready for extra chemical work. After that, the treated sugarcane bagasse (SCB) was able to get back chemically clean cellulose (CNCs) and cellulose nanocrystals (CPC). These results back up what a previous study found: SCB could be a cheap and renewable source of cellulose, especially for biomedicine and sustainable materials (Evans et al., 2019). The pretreatment procedure utilized in this study performed effectively to acquire more cellulose and make the results more reliable.

6.2 Submerged Fermentation for bacterial Growth on SCB:

SCB is industrial wastes were used in submerged fermentation (SmF) to improve yield and reduce production costs. During SmF, both biomass types began to exhibit an orangish coloration, suggesting bacterial growth. Colony count analysis showed a growth trend with a logarithmic phase from 0 to 24 hours, followed by a stationary phase up to 48 hours. After this point, microbial growth declined due to nutrient depletion in the batch reactor. The substrates used not only worked as inert support but also were utilized as nutrient for bacterial growth and vitamin production bestowing eminent productivity as no additional source of carbon was used. In this batch culture bacteria utilize the SCB as a carbon source in liquid media which is YPMSM. (FIG 1)

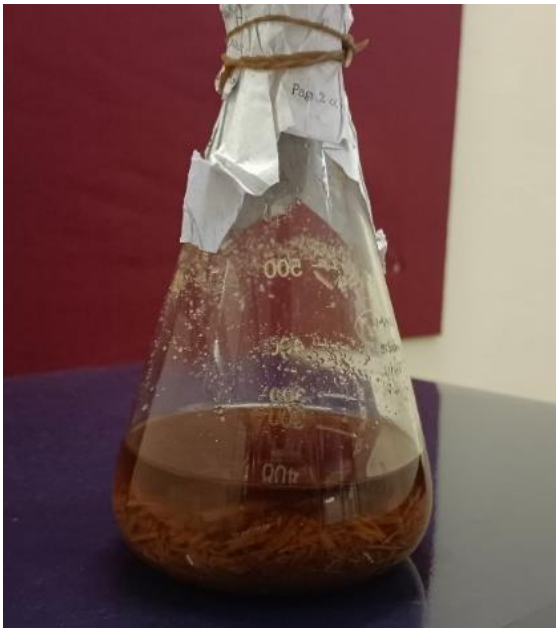


FIG 1

6.3. Growth kinetics –

We counted the number of colony-forming units (CFUs) every so often throughout a 7-day incubation trial with Yeast- Peptone Mineral Salt Medium (YPMSM) and Sugarcane Bagasse (SCB). This let us keep track of how quickly the bacteria were developing.

After 24 hours on Day 1, there was no early growth, but after a longer incubation period, the colonies started to grow. This means that there were germs that were either dormant or developing slowly.

There were only 9 colonies on the 10^{-6} diluted plate on Day 2. This showed that the bacteria were starting to grow.

A lot more bacteria grew on Day 3. The pure material created 395 colonies, and the 10^{-6} dilution made 300 colonies, both of which were yellow-orange. This shows a fast exponential development phase, and the pigment generation may be linked to certain types of bacteria.

At the 10^{-4} dilution, the number of colonies dropped to 63 on Day 4. This could have happened because there weren't as many germs in the inoculum or because the environment made it hard for things to thrive. Even after 48 hours, there were no colonies on Day 5. This may mean that the bacteria were dying or that the conditions for colony formation were no longer good.

Day	Dilution Factor	Incubation	Observation (colony count)	Colony Morphology
1	Undiluted	36°C for 24h (extended to 48h)	No colonies after 2 Numerous colonies after 48h	Not specified
2	10^{-6}	36°C for 24h	9 colonies	Yellowish-orange pigment
3	10^{0} & 10^{-6}	36°C for 24h	Undiluted: 395 colon Diluted: 300 colonies	Yellowish-orange pigment
4	10^{-4}	36°C for 48h	63 colonies	Yellowish-orange pigment
5	10^{-4}	36°C for 48h	O colonies	Not specified

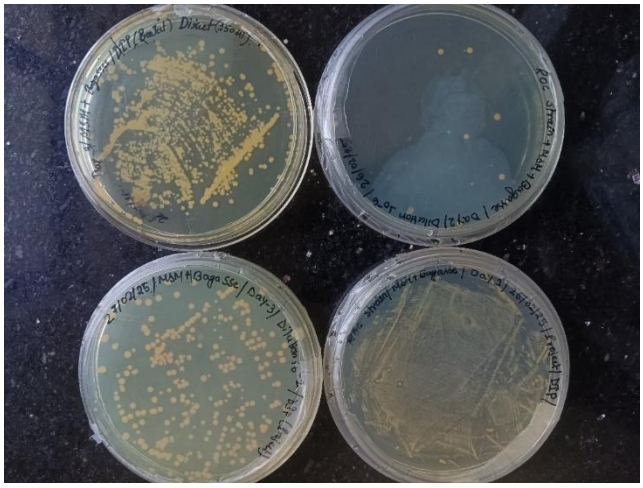
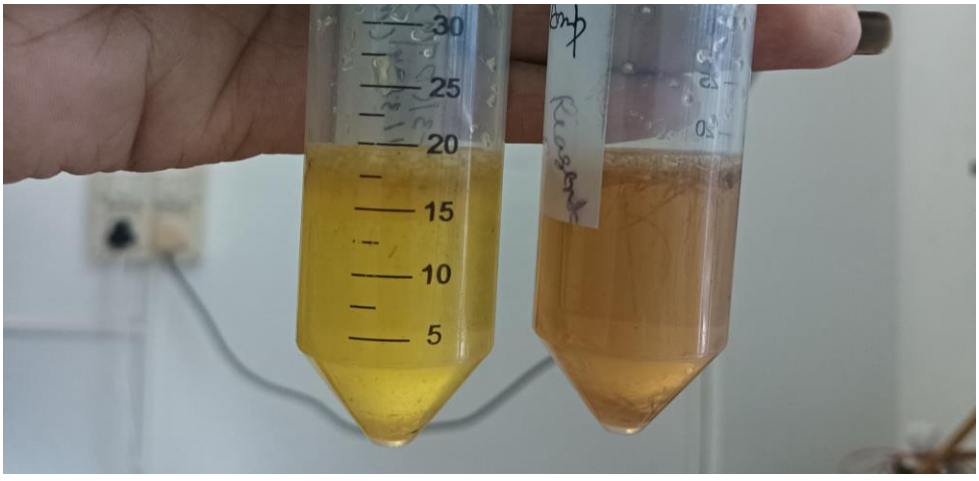


FIG 2

6.4. Assessment of LCB Deconstruction: -

For Alkali lignin - Using NaOH to pretreat lignocellulosic biomass like sugarcane bagasse is a cheap, effective, and scalable way to break down alkali lignin. It removes a lot of lignin, smooths out the surface, and makes more cellulose available. NaOH breaks down the ester and ether bonds in lignin and hemicellulose very well. This makes the cellulose simpler to get to by making the fibers and pores bigger. We wanted to see if bacteria could live and thrive in sugarcane bagasse (SCB). To do this, we grew yellow-orange pigment-producing bacteria in Yeast Peptone Mineral Salt Medium (YPMSM) with both treated and untreated SCB. We evaluated the optical density (OD) at 600 nm after the samples had been in the incubator. The culture with NaOH and treated SCB had an OD465 of 0.402, which is higher than the culture with untreated SCB, which had an OD465 of 0.359. This means that the bacteria did better in the initial culture. Adding alkali to SCB made it easier for bacteria to grow since it made more substrate available. The SCB sample that had been treated with NaOH had an optical density (OD) of 0.402, while the SCB sample that had not been treated had an OD of 0.359. The lignin degradation formula gave a negative degradation value of –11.98%, which means that the treated sample had more lignin that could dissolve than the untreated sample. The rise in OD following alkali treatment suggests that NaOH broke apart the lignocellulosic matrix, which made it easier for alkali lignin to dissolve in the liquid phase. It is likely that the alkaline circumstances destroyed the ester and ether bonds between lignin and other structural carbohydrates, especially hemicellulose, which let it out. Other research have discovered the same things: NaOH treatment is very good in breaking apart complexes of lignin and carbohydrates. This makes it easy to throw away things like SCB that come from farming.

Parameter	Untreated SCB with NaOH	Treated SCB with NaOH	Significance
Color	Yellowish to light brown	Dark brown to pale yellow	Indicates lignin removal— darker color in black liquor = more lignin solubilized
Weight loss (%)	~5–10% (due to minor drying)	30–50% (due to loss of lignin hemicellulose)	Quantifies solubilized non-cellulosic components
Lignin content (%)	20–30%	Reduced to 5–10%	Shows effective lignin removal
Cellulose content (%	~35–40%	Increased to ~50–65% (relative %)	Improved enzymatic digestibility and microbial access
Hemicellulose con (%)	~25–30%	Decreased to ~10–15%	Partial solubilization under alkaline conditions



Untreated with NaOH **Treated with NaOH**

FIG 3

Acid soluble and insoluble lignin: -

The two-step acid hydrolysis method with sulfuric acid (H_2SO_4) works effectively to separate the acid-insoluble lignin (AIL) and acid-soluble lignin (ASL) parts of sugarcane bagasse (SCB). When you use concentrated H_2SO_4, it is easier to break up lignin–carbohydrate complexes. This makes cellulose and hemicellulose more available since it breaks the structural link between them and lignin. This break is highly critical for making the next steps in hydrolytic or microbiological breakdown work better. We utilized a UV-Visible spectrophotometer to measure how much light the filtrates absorbed at 320 nm. This was a way to get a number for the acid-soluble lignin concentration. The optical density (OD_{320}) of the treated SCB sample was 1.596, which is a bit lower than the 1.653 seen in the control sample that wasn't treated. Absorbance going down suggests that there are fewer aromatic molecules that can absorb light. This means that the H_2SO_4 pretreatment worked and broke down some of the lignin, specifically the ASL part. The drop in measurements corresponds to an estimated ASL degradation efficiency of 3.45%. This means that the amount of soluble lignin dropped after treatment, even though it was only a little amount. This drop shows that acid treatment not only modifies the structure of lignin, but it also helps to get rid of some of the soluble lignin parts. The acid pretreatment had a higher effect on the lignin fraction that doesn't dissolve in acid than on the ASL content. The residual solid material from treated SCB weighed 0.2633 g when it was dry. This is a substantial decline from the 0.7501 g seen in the untreated sample. This means that AIL has gone down by 64.88%, which means that the tough lignin structures have been broken down. The big decline in AIL content demonstrates that the microbial strain used in the study worked since it appeared to have broken down or chemically altered a lot of the lignin matrix during incubation. The big decline in both OD values and residual solid mass suggests that a lot of lignin has already been broken down and dissolved before the acid hydrolysis process. This highlights how vital it is to treat biomass with microbes before it is digested. This is especially useful for downstream bioconversion activities, where intact lignin might make it impossible for enzymes or microorganisms to access to cellulose and hemicellulose. The microbial strain was particularly adept at breaking down the AIL part in a selective way. This got rid of structural obstacles that would have made enzymatic saccharification and fermentation less effective.

Parameter	Untreated SCB with H2So4	Treated SCB with H2SO4	Significance
Acid-Insoluble Lignin	High (~18–25% of dry weight)	Lower (~7–10%)	Indicates significant removal structural lignin (core delignification)
Acid-Soluble Lignin	High	Lower	Reflects solubilization of lignin fragments into hydrolysate
Total Lignin Content	~20–27%	~10–15%	Overall lignin reduction (~40–60%) supports effective pretreatment
Color of Hydrolysate	Dark brown	Light brown	Darker color indicates dissolved lignin fragments (ASL)

Untreated with H2SO4 **Treated with H2SO4**

FIG 4

Difference in hemicellulose and cellulose content-

We looked at the dry biomass that was left over after alkaline treatment to see how well we could get hemicellulose out of sugarcane bagasse (SCB). The SCB sample that was treated with 500 mmol/L NaOH had a final dry weight of 0.223 grams. The SCB sample that wasn't treated had a lower residual weight of 0.157 grams. These data tell us how much biomass was left after the hemicellulose was broken down and how much hemicellulose was removed throughout the treatment. We found out how much hemicellulose was taken out by weighing the sample before treatment (1.000 grams) and then weighing it again after treatment. The treated SCB still had 0.777 grams of hemicellulose in it, which means that 77.7% of it was removed. The untreated SCB sample, on the other hand, lost 0.843 grams of hemicellulose, which means that 84.3% of it was removed. It's intriguing that the untreated sample showed a little higher percentage of hemicellulose extraction. The acid pretreatment might have modified the ratio of lignin to hemicellulose Taking out or moving lignin around during pretreatment may have made hemicellulose and cellulose interact more strongly, making hemicellulose less soluble in alkaline solutions. Another possibility is that the changes to the structure that happened during pretreatment might have generated hemicellulose derivatives that are less prone to break down chemically. These data indicate how difficult it is to break down lignocellulosic biomass (LCB). For instance, actions taken before treatment can assist remove lignin, but they may also make it difficult to remove other polymers, such as hemicellulose. Biorefineries that wish to make the most fermentable sugars or other valuable products need to know these things so they can improve how they process things from the start.

Reducing Sugar Estimation via DNS Assay

The dinitrosalicylic acid (DNS) method was used to figure out how much reducing sugars were in the lignocellulosic biomass (bagasse). This method is based on the idea that when reducing sugars are mixed with DNS reagent at high temperatures, they generate a colorful complex that can be detected using a spectrophotometer. making a colored complex that can be seen at 540 nm. In this work, glucose was employed as the standard, and a calibration curve was created for concentrations between 0.2 and 1.0 mg/mL. The table below shows the results of the standard readings:

Concentratiion	OD
0.2mg/ml	0.083
0.4mg/ml	0.149
0.6mg/ml	0.164
0.8mg/ml	0.207
1mg/ml	0.258
1ml Bagasses supernatent	0.175

The relationship between concentration and absorbance was found to be linear, confirming the validity of the assay in this range. The regression equation derived from the standard curve was: The intensity of the resulting orange-red color is directly proportional to the concentration of reducing sugars present in the sample. A linear regression analysis was conducted on the standard data to obtain the equation of the best-fit line, following the format y = mx+c

where:

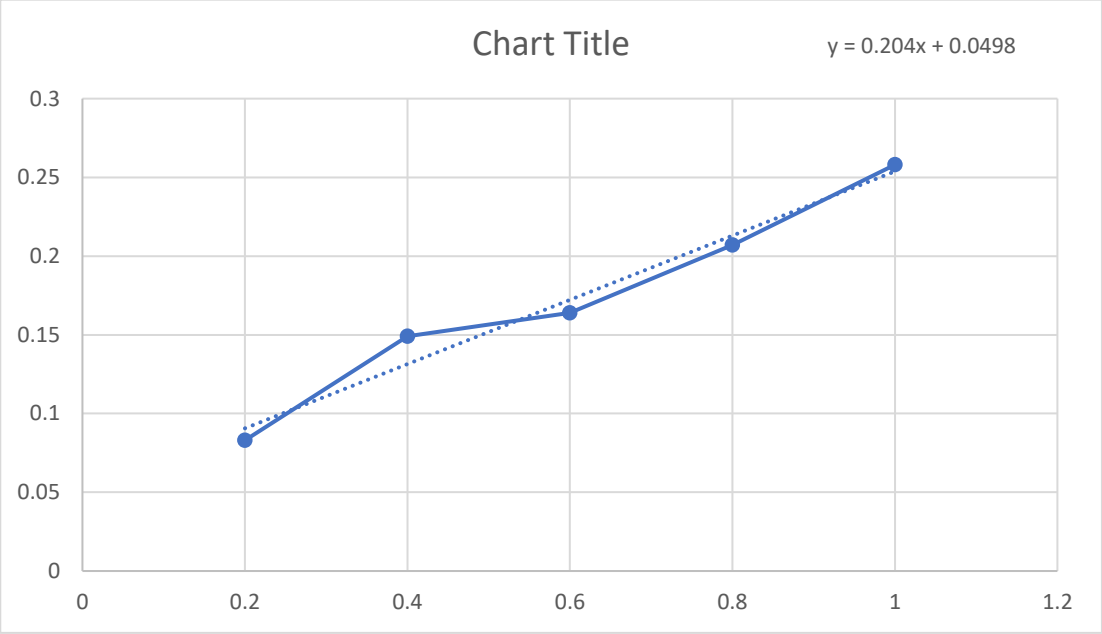
- y is the optical density (OD),
- x is the glucose concentration (mg/mL),
- m is the slope,
- c is the y-intercept.

The absorbance of the unknown sample (bagasse supernatant hydrolysate) was found to be 0.175. Substituting this value into the regression equation:

$$x = \frac{y-c}{m} = \frac{0.175-0.049}{2.204} = 0.617647 mg/ml$$

The optical density of the hydrolyzed bagasse sample is 0.175. The regression equation told us that the sample had roughly 0.617647 mg/ml of reducing sugar. This suggests that hydrolysis freed a lot of sugars that could be fermented from the biomass. Bagasse is the leftover stuff from refining sugarcane. It is mostly made up of cellulose, hemicellulose, and lignin. It is likely that the hydrolysis process broke down the cellulose and hemicellulose parts into simpler sugars, according

to this study. Bagasse gives off reducing sugars, which implies it might be a viable raw material for generating bioethanol and other valuable things in the biorefinery business. The DNS method works effectively and always delivers the same results, thus it's a suitable alternative for regularly testing biomass sugars. But it's important to realize that the DNS method only yields an estimate of the amount of reducing sugars, not the overall amount of carbs. This means that the full sugar level may be a little lower than it really is if oligosaccharides are present. The possibility of using agro-waste, such bagasse, in sustainable biochemical manufacturing. This will enable us come up with better ways to use bioresources and make trash more valuable.

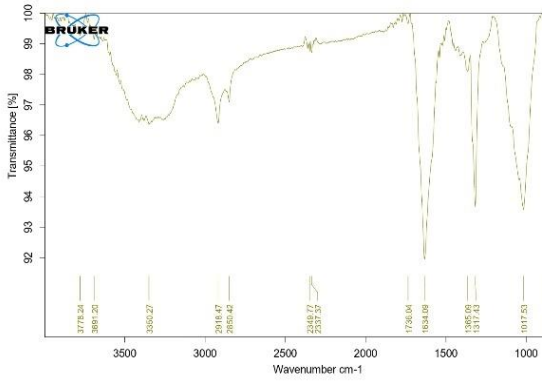


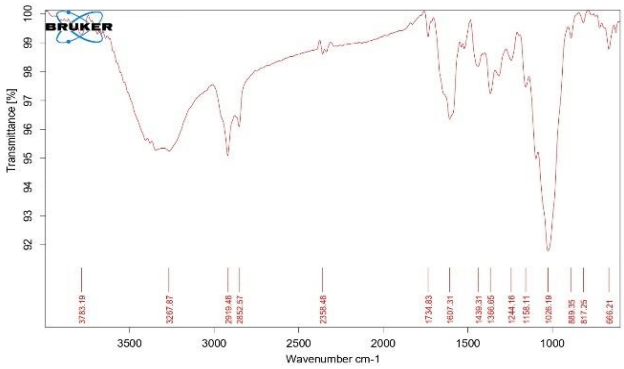
Standard curve of sugar estimation

FIG 5

6.5 Physiochemical Characterization of Deconstructed SCB:

- **Fourier transform infra-red spectrometry (FTIR)analysis –**





(FIG 6) Untreated SCB FTIR Analysis **(FIG 6.1) Treated SCB FTIR Analysis**

Both of these describe FTIR peaks relevant to lignocellulosic biomass but focus on different functional groups and spectral regions. The first paragraph highlights peaks associated with aromatic C–H bending and carbonyl (C=O) stretching around 1734 cm^{-1}, O–H bending from carboxylic acid groups near 1439 cm^{-1}, and C–N stretching indicative of amine groups at about 1026 cm^{-1}, emphasizing structural components like lignin, hemicellulose, and nitrogen-containing residues or chemical modifications. In contrast, the second paragraph discusses peaks linked to C=C stretching vibrations at 1634 cm^{-1} typical of lignin's unsaturated aromatic structures, an uncommon C–F stretching peak at 1017 cm^{-1} indicating possible fluorinated additives or contamination, and O–H bending vibrations around 1365 cm^{-1} related to hydroxyl groups abundant in cellulose and hemicellulose. Together, they provide complementary insights into the chemical complexity, modifications, and composition of lignocellulosic materials by focusing on different molecular vibrations and their associated biopolymer components. FTIR analysis of Treated and untreated SCB that has been already reported in (Mandal, 2011).

6.6 Biopigment Extraction :

We made a fermentation system with Yeast Peptone Mineral Salt Medium (YPMSM) and 20% (w/v) sugarcane bagasse (SCB) as the carbon source so that we could manufacture and get microbial vitamins. We put the medium into Erlenmeyer flasks that held 500 mL apiece and then autoclaved them to destroy any microorganisms. 5% of a bacterial inoculum that had developed overnight was introduced after sterilization without any contamination. For 7 days, the mixture was stirred constantly at 120 rpm while it was submerged in 30 °C water. We screened the whole culture after the incubation time to get rid of any particles that were still there. We next spun a section of the culture broth at 8000 rpm for 15 minutes to separate the supernatant from the bacterial cells. After that, the biomass was used to acquire vitamins. The culture was spun again at 8000 rpm for 10 minutes at 4°C to generate a tight cell pellet. We cleaned this pellet and then put it back in methanol, which is a wonderful way to produce vitamins that dissolve in both water and fat. The suspension was spun in a centrifuge to get rid of any extracellular material that was still there after it had been mixed gently. Sonication of the methanol-treated suspension broke down the cells completely and freed the vitamins inside. The ultrasonic treatment rendered the walls of the bacterial cells weaker, which helped the extraction operate better. The lysate was spun again at 8000 rpm for 10 minutes after sonication. The supernatant that came out of this process, which had the vitamins in it, was collected and stored at low temperatures for further examination using UV-Vis spectrophotometry or HPLC.

6.7 Characterization of Biopigments-

- **Fourier transform infra-red spectrometry (FTIR)Analysis –**

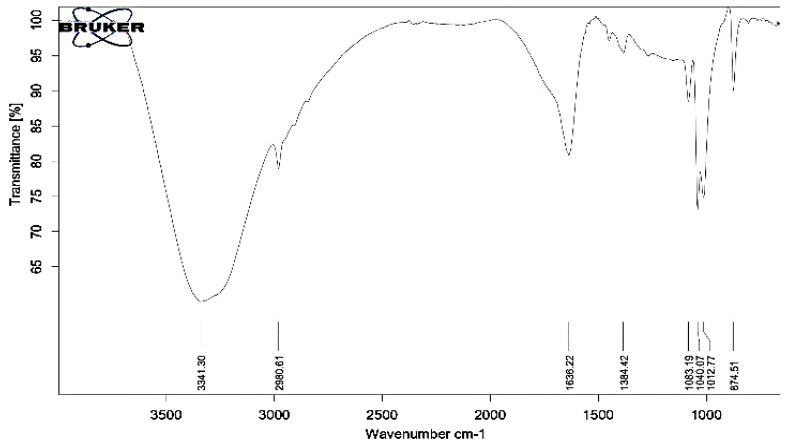


Fig 7. FTIR analysis of Riboflavin (vitamin b2)

The FTIR signal at 3341.30 cm^{-1} in vitamin extracts is often linked to O–H stretching vibrations, which means that hydroxyl groups are present. This peak seems wide and strong, which is what you would expect from alcohols that form hydrogen bonds. Its presence shows that there are alcohol functional groups, which are common in vitamins like ascorbic acid (Vitamin C). The broad form comes from hydrogen bonds between molecules, which is something that polar substances do. This part of the spectrum is significant for finding alcohol-class chemicals in natural product extractions. The other FTIR signal at 1083.19 cm^{-1} that is detected in vitamin extracts is often associated to C–F stretching vibrations, which means that fluoro functional groups are present. This peak is usually crisp and has a moderate to strong intensity, which is what you would expect from fluorinated organic compounds. The fact that it is there suggests that fluoro compounds may be present, maybe coming from fluorinated derivatives or additives employed in the vitamin's formulation or extraction process. C–F bonds usually absorb light in the 1000–1400 cm^{-1} range, and a peak about 1083 cm^{-1} helps to confirm the presence of fluorinated groups in the sample that was examined. The FTIR peak at 1636.22 cm^{-1} seen in vitamin extracts is usually associated to C=O stretching vibrations, which show that carbonyl groups are present. This range is common for secondary amides, which have a nitrogen atom linked to a carbonyl group. The peak usually seems robust, but it may be a little off because of things like hydrogen bonding or conjugation. Finding it means that secondary

amide molecules are present, which may have come from protein-related chemicals or certain vitamin derivatives. The 1636 cm^{-1} area is very important for finding amide groups in complicated mixes like vitamin extracts.

- **UV-Vis spectroscopy Analysis:**

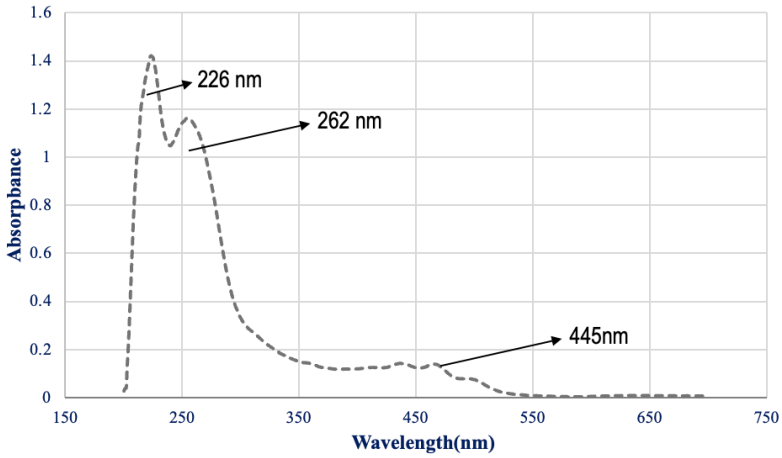


FIG 8: UV-VIS Scan Analysis of Riboflavin (Vitamin b2)

Recent studies have shown that lignocellulosic hydrolysates can provide riboflavin (vitamin B2) for a long time. Most people know that riboflavin has very high absorption peaks in its UV-Vis spectrum. SIELC Technologies says that the absorption maxima that are most important are 224 nm, 268 nm, 370 nm, and 446 nm. The UV-Vis spectrum has a major peak at 223 nm, where the value is 1.45. The shoulder is at 265 nm, and the absorbance is about 1.15. The x-axis of the graph shows wavelengths from 200 to 750 nm, which is the range of both UV and visible light. The y-axis, which shows how much light is absorbed, goes from 0 to 1.6. The spectrum shows that absorbance reduces quickly after 300 nm and stays low in the visible range (400–750 nm). It makes logical that riboflavin has a pale-yellow tint. The $\pi \rightarrow \pi^*$ electronic transitions that make the big peak at 223 nm are often linked to the aromatic and conjugated structures of the riboflavin isoalloxazine ring. The $n \rightarrow \pi^*$ transitions are most likely what generated the shoulder at about 265 nm. This happens when lone pair electrons move from one element to another, such oxygen or nitrogen. There are no extra peaks in the spectral profile that would signal that something is wrong or that it has become worse. This proves that riboflavin is there and is clean. UV-Vis spectrophotometry is a good way to find and analyze riboflavin in both living things and enterprises. The pH of the solution also affects how riboflavin absorbs light (Bartzatt 2014) indicates that the peaks stay in the same places between pH

3.02 and 9.80, but the molar absorptivity can shift. When the pH was 5.03 (citric acid buffer), the molar absorptivity at 440 nm was 11,772.7 $L \cdot mol^{-1} \cdot cm^{-1}$. This makes it easier to study the molecule and makes it more stable.

7. Conclusion –

This study demonstrated that a pigmented indigenous bacterial monoculture (ROC1) can break down lignocellulosic biomass like sugarcane bagasse (SCB). The bacterial strain did very well in a fermentation tank that simply used sugarcane bagasse and a low-salt medium as its source of carbon. The tests that focused on breaking down lignin and generating colors that are excellent for health produced the best results. The NaOH alkali pretreatment made the cellulose easier to get at and lessened the amount of lignin, which sped up the process of breaking it down. There was a 64.88% drop in the amount of acid-insoluble lignin (AIL). This means that the chemical preparation and bacterial treatment might work well together to break down the lignocellulosic matrix, which is hard to perform. This is a big step toward making it easier to break down biomass for bioprocessing. Researchers employed FTIR and UV-Visible spectroscopy to find out that SCB breaks down into riboflavin (vitamin B2), which has a beneficial yellow-orange color. Riboflavin is important for both your health and your business. Making items using microbes is healthier for the environment than making them with chemicals. There were obvious peaks at 223 nm and 265 nm in the UV-Vis spectra, which suggests that riboflavin was there. FTIR examinations showed that there were functional groups such amide, hydroxyl, and carbonyl. The DNS test showed that bacteria might be able to use SCB since it released reducing sugars, which suggests that it broke down cellulose and hemicellulose. It has roughly 0.617 mg/mL of sugar in it, therefore it can transform a lot of substances into sugar. This means that it can easily get carbon from SCB. This test indicates that you can generate vitamins and waste feedstock at the same time. It's incredible that anything can produce money and be good for the environment at the same time. ROC1 is a form of bacteria that can grow on its own. It seems to be useful for bioindustrial uses that use biomass to generate riboflavin. Researchers may look for ways to improve this process for larger fermentation and green biorefinery systems in the future.

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1. Abstract-

Sugarcane bagasse is an abundant agro-industrial lignocellulosic biomass with great potential as a renewable raw material for sustainable bioconversion. In this study, we investigate the microbial degradation of sugarcane bagasse by a naturally occurring, pigmented bacterial strain (ROC1) isolated from soil. Bagasse is rich in cellulose, hemicellulose and lignin and is a good candidate for microbial treatment to produce value added products. The bagasse was pretreated with sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4) to pretreat the bagasse to enhance the bioavailability of its fibrous components by breaking down the rigid structure. Subsequently, the treated biomass was used as the sole carbon source in a submerged fermentation setup with yeast-peptone mineral salt medium. Colonization and rapid proliferation of the bacteria was confirmed by monitoring bacterial growth through colony forming unit (CFU) counts and optical density. The degradation efficiency was assessed by DNS assays and UV-Vis spectrophotometry which confirmed significant breakdown of lignin, cellulose and hemicellulose. Interestingly, the bacterial strain also produced a yellow orange pigment which was identified as riboflavin (vitamin B2) by FTIR and UV-Vis spectroscopy. FTIR further structural analysis showed significant chemical changes in the sugarcane bagasse matrix after bacterial treatment. These results indicate that native soil bacteria can degrade lignocellulosic waste and produce valuable bioproducts simultaneously. Sugarcane bagasse is positioned in the study as a waste byproduct, but also as a promising substrate for microbial fermentation and green bioprocessing applications.

Keywords:-[sugarcane bagasse, lignocellulosic biomass, bacterial monoculture, riboflavin, biopigment, submerged fermentation, biodegradation, waste valorization]

2. INTRODUCTION -

Lignocellulosic biomass (LCB) comes from plant material, is plentiful and renewable and contains three primary polymers: cellulose, hemicellulose and lignin (Zoghlami 2019). The structure of LCB suggests it will be a good and economical source of fermentable sugars and other useful biochemicals. Even so, biodegrading LCB is tricky because it involves many different types of enzymes (Tolalpa, 2017). Out of all lignocellulosic residues, sugarcane bagasse (SCB) has stood out as a favorable option for bioconversion techniques. SCB is a waste material that forms when sugarcane juice is pressed and there is a lot of it in places with tropical and subtropical climates. For this study, the SCB came from a local juice vendor in Barasat and was used because it was cost-friendly and easily available. The major building blocks of SCB are cellulose, hemicellulose and lignin, of which Xylan accounts for most of the hemi cellulosic part, while glucan includes both cellulose and hemicellulose (Vieira., 2019). Researchers isolated a gram-positive, pigmented bacterium from a soil sample in Kolkata, India and

used it to help microbes degrade SCB. According to Nunes (2019), the decomposition of cellulose, hemicellulose and lignin in LCBs is not the same for every microorganism due to their different enzymatic and biochemical skills. A number of bacterial strains are recognized for breaking down lignocellulose and producing riboflavin (vitamin B2) which is a commercial product (Dzanaeva., 2024). Among its many jobs, riboflavin helps by making FMN and FAD which are important parts of cellular metabolism. They are necessary for important chemical changes during the metabolism of carbohydrates, lipids and proteins. While riboflavin was synthesized by chemical means previously, it is now made more often by fermentation since it is cost-friendly and environmentally sustainable. Significantly, Bacillus subtilis is used in industrial riboflavin manufacturing, but the strong cellulolytic abilities of B. coagulans, B. licheniformis and B. cereus enable these strains to degrade cellulose and hemicellulose (Kumar, 2024). Thermomonospora fusca and Cellulomonas fimi together play important roles in breaking down lignocellulose. The composition of sugarcane bagasse is 45-50% cellulose, 25-30% of hemicellulose, and 25% lignin (S. Niju 2019)

Because SCB is a waste product from farming, it can be used in various industries thanks to its high amount of cellulose. The objective of this investigation was to separate out cellulose nanocrystals (CNCs) and chemically purified cellulose (CPC) from SCB to show its potential use in biomedical applications (Evans, 2019). An initial microbial pre-treatment step in YPMSM was applied to SCB, as it contains important phosphates, nitrates, sulphates and chlorides that microbes need without added carbon (Park, 2020). Many biodegradation experiments use YPMSM because it promotes microbial growth and obliges them to feed on lignocellulosic materials only (Kaur, 2021). A nutrient broth culture of each bacteria was put in an incubator at 36°C and agitated at 120 rpm for 12 to 16 hours before commencing. The bacteria were added at a 5% concentration to the YPMSM with SCB and incubated at 30°C for 120 hours, with mixing done every 12 hours. R. Khaneja (2009) also considered the effect of waste biomass on pigment production. Submerged fermentation was selected over solid-state fermentation because controlling temperature, pH and oxygen is easier with submerged fermentation (Pyter, 2022). Solid-state microbial fermentation ensures equal bacterial growth, increases the amount of produced products and provides an easy scaled process for industry purposes. What's more, SmF speeds up extracting useful items since products can be extracted from the liquid phase without complications. To evaluate the results of microbial treatment on SCB, analytical and biochemical methods were used. To improve the breakdown of biomass, solutions of sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4) were used according to methods described in previous investigations (Anwar, 2014). Biomass that had been treated was studied using FTIR spectroscopy and UV-Visible spectrophotometry to understand both its structure and its a chemical changes (Majumdar, 2020).

3. Objective

- To perform submerged batch fermentation of bacterial strain in SCB containing Yeast-peptone mineral salt media
- To assess the LCB deconstruction potential of a given bacterial monoculture – strain ROC1
- To extract and analyse the coloured bio pigment produced by the strain.

Plagiarized Sources

Significantly, Bacillus subtilis is used in industrial riboflavin manufacturing, but the strong cellulolytic abilities of B.
https://pmc.ncbi.nlm.nih.gov/articles/PMC10146818

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